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论著

RNA干扰阻抑Bax inhibitor-1(bi-1)基因表达对卵巢癌细胞凋亡的影响

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摘要 目的： 研究RNA干扰(RNAi)效应对人卵巢癌细胞株HO8910PM和HO8910中bi-1基因表达的抑制作用和凋亡诱导作用。 方法： 把表达bi-1 shRNA的各重组干扰质粒转染到HO8910PM和HO8910细胞中，RT-PCR和Western blotting法检测bi-1基因mRNA和蛋白表达水平；流式细胞术和Hoechst 33258/PI荧光染色检测siRNA诱导细胞凋亡作用。结果：与对照组细胞相比较，转染4种候选干扰质粒组细胞中bi-1的mRNA和蛋白表达水平均明显下降（P<0.05），以转染pmU6-b4组细胞的表达抑制率最高，在HO8910PM细胞中的抑制率分别为（45.50±3.04）%和(51.08±4.96）%，在HO8910细胞中的抑制率分别为（37.29±3.69）%和(44.96±4.28）%；流式细胞术结果表明，与对照组细胞相比较，转染pmU6-b4质粒24 h、48 h、72 h、96 h的HO8910PM细胞中亚二倍体峰比值明显升高（P<0.05)，以转染72 h组细胞的比值最高，达到(25.89±5.63）%，而HO8910细胞中无亚二倍体峰出现；荧光显微镜下可见转染pmU6-b4质粒72h的HO8910PM细胞出现细胞核缩小、染色质固缩和核断裂，而HO8910细胞核无明显变化。 结论： 针对bi-1的siRNA能特异有效地下调HO8910PM和HO8910细胞中bi-1基因的表达，不同序列特异性的siRNA下调bi-1基因表达的能力不同；瞬时转染质粒pmU6-b4能有效诱导HO8910PM细胞凋亡，但不能诱导HO8910细胞凋亡。

关键词 Bax 抑制剂-1； RNA干扰； 卵巢肿瘤； 细胞凋亡

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Ovarian serous cystadenocarcinoma cell apoptosis induced by inhibition of Bax inhibitor-1 (bi-1) gene expression with RNA interference

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Abstract

<FONT face=Verdana>AIM: To study the inhibition of the Bax inhibitor-1 (bi-1) gene expression induced with RNA interference in HO8910PM and HO8910 cell lines in addition to the apoptotic induction. METHODS: After transfection of recombinant plasmid which expresses bi-1shRNA into HO8910PM and HO8910 cells, bi-1 mRNA and protein levels were detected by reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting. Apoptotic HO8910PM and HO8910 cells were detected by Hoechst 33258/PI fluorescent staining and flow cytometry. RESULTS: Compared with the control group, the expressions of bi-1 gene at mRNA and protein levels were declined evidently in the cells transfected with four candidate siRNA plasmids (P<0.05). The pmU6-b4 plasmid transfected group had the most efficient inhibition effect, the inhibition rate of mRNA and protein was (45.50±3.04）% and (51.08±4.96）% in HO8910PM cells, (37.29±3.69）% and (44.96±4.28）% in HO8910 cells, respectively. After treated with pmU6-b4 plasmid for 24 h, 48 h, 72 h, 96 h, the percentage of apoptotic cells in sub-G1 phase was significant increased in HO8910PM cells compared with that in the control group cells (P<0.05). Flow cytometry analysis showed that about (25.89±5.63) % of HO8910PM cells were apoptotic at 72 h after transfection. However, no sub-G1 phase in HO8910 cells was observed. Cell shrinkage, chromatin condensation, and

nuclear fragmentation were found under fluorescent microscope after treated with pmU6-b4 plasmid for 72 h in some HO8910PM cells, but was not in HO8910 cells. CONCLUSION: The siRNAs targeted against bi-1 in vitro are able to decrease the expression of bi-1 with different capabilities of the specific siRNAs down-regulation. The transient transfection of pmU6-b4 effectively induces apoptosis in HO8910PM cells, but could not induce HO8910 cell apoptosis.</FONT>

Key words Bax inhibitor-1 RNA interference Ovarian neoplasms Apoptosis

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